

GENETIC RELATIONSHIPS AMONG *FREMONTODENDRON*
(STERCULIACEAE) POPULATIONS OF THE CENTRAL SIERRA NEVADA
FOOTHILLS OF CALIFORNIA

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ABSTRACT

Fremontias, or flannel bushes (*Fremontodendron*), are a distinctive element of California's chaparral communities. *Fremontodendron decumbens* is only known from a few populations in gabbro soil plant communities of the Sierra Nevada foothills in El Dorado County. Although a recovery plan for these communities has been drafted, the long-term management of *F. decumbens* is complicated by its treatment as a subspecies of the more widespread *F. californicum*, and by the recent discovery of additional populations of decumbent plants in Yuba and Nevada Counties that are not easily assigned to either *F. californicum* or *F. decumbens*. Genetic relationships among 5 populations, including *F. californicum*, *F. decumbens*, and the decumbent plants in Yuba County, were ascertained using AFLP markers. Principal coordinates and population structure analyses of the AFLP data showed that *F. decumbens* is genetically distinguishable from the populations of *F. californicum* that we sampled. This distinction, coupled with its unique morphology and ecology, support the treatment of *F. decumbens* as a species and promote its continued conservation as a rare and unique element of plant communities on gabbro soils in the Sierra Nevada. The decumbent Yuba County population shared a number of alleles with *F. californicum* and *F. decumbens* and the analyses did not clearly distinguish its taxonomic relationships. It is possible that this population represents an historical hybrid between *F. californicum* and *F. decumbens*. A resolution of the taxonomic position of the decumbent Yuba County populations will require more thorough sampling of *F. californicum* but the presence of unique alleles in this population suggests that it also should be conserved.

Key Words: *Fremontodendron*, AFLPs, STRUCTURE, population genetics, conservation, gabbro soils.

The flannel bushes or fremontias (*Fremontodendron*) are a distinctive element of chaparral vegetation in the Sierra Nevada foothills and coastal ranges of California, extending to scattered locations in central and western Arizona and the northern Baja California of Mexico. Kelman (1991) recognized three species: *Fremontodendron californicum* (Torrey) Coville, *F. mexicanum* Davidson, and *F. decumbens* R.M. Lloyd. *Fremontodendron californicum* is the most widespread and polymorphic, while *F. mexicanum* is confined to southern California and the Baja California peninsula of Mexico. *Fremontodendron decumbens* is an endemic element of the unique gabbro soil plant communities of the Sierra Nevada foothills, and was described from a population on the Pine Hill formation in El Dorado County (Lloyd 1965). It is known from only a few populations at this site, and was listed as federally endangered in 1996 (USFWS 1996) and included in a recovery plan for gabbro soil plants of the central Sierra Nevada foothills in 2002 (USFWS 2002). The long-term management of *F. decumbens* as an endangered species is complicated by two issues regarding its taxonomic rank: firstly by the treatment of *F. decumbens* as a subspecies of

the more widespread *F. californicum* in the Jepson Manual (Hickman 1993), the primary reference for the California flora; and secondly by the discovery of other decumbent populations of *Fremontodendron* that are not assignable to *F. decumbens* on morphological grounds.

Diagnostic features of *F. decumbens* are its decumbent growth habit, orange to copper-red flowers (as opposed to the yellow flowers of *F. californicum* and *F. mexicanum*), dense stellate pubescence with long trichome rays on the abaxial leaf surface, and long peduncles. Kelman (1991) noted the presence of *F. decumbens* plants 1 km from the population at the summit of Pine Hill, and a large population of *F. decumbens* is also present on the eastern boundary of the Pine Hill Ecological Reserve (Boyd 2003). Beyond the Pine Hill site, decumbent plants of *Fremontodendron* have been collected from a number of localities in Yuba, Butte, and Nevada Counties. G. L. Stebbins collected plants from a population at a city dump site near Dobbins, Yuba County in 1966. An occurrence of decumbent *Fremontodendron*, with "deeper orange and smaller and more campanulate" flowers was noted by Campbell (1980) at a roadside locality near Campton-

TABLE 1. MEAN AND STANDARD ERROR (IN BRACKETS) FOR DIAGNOSTIC MORPHOLOGICAL CHARACTERS OF *FREMONTODENDRON* POPULATIONS. ¹Data from Kelman (1991), ²Measurements of 10 shrubs grown at Canberra, Australia, ³Basal branches well developed, plants as wide as tall.

	<i>F. californicum</i> ¹	<i>F. decumbens</i> ¹	<i>F.</i> "Yuba County" ^{2,3}
	n = 49	n = 48	n = 10
Habit	erect	decumbent ³	decumbent
Leaf length (mm)	42 (9)	32 (8)	27 (6)
Leaf width (mm)	25 (10)	25 (7)	27 (10)
Trichome ray length (mm)	0.34 (0.19)	0.76 (0.14)	0.47 (0.10)
Flower color	yellow	orange to mostly copper-red	orange
Flower diameter (mm)	46 (8)	41 (6)	26 (5)
Peduncle length (mm)	9 (2)	17 (4)	6 (2)

ville, Yuba County. In 1975, Lowell Ahart collected plants from a population at a dump site near Brownsville, Yuba County. Later observations of this population indicated that although the plants were uniformly decumbent, the pale orange to yellow flower color and shorter peduncles argued against their assignment to *F. decumbens* (Table 1). In Butte County, Ahart collected plants from a site on the Bloomer Road, east of Lake Oroville. This population was described in the Manual of Vascular Plants of Butte County as "low and stunted but apparently not the ssp. *decumbens* (R.M. Lloyd) Munz ... known only from Eldorado and Nevada counties". In 1985, a small population of decumbent plants was reported near Grass Valley in Nevada County (Marcia Braga personal communication). Using the diagnostic characters, these plants were not assignable to *F. decumbens* in that flower color was not as deep orange and the peduncles and trichome rays were not as long as those in *F. decumbens* measured by Kelman (1991) (Table 1). Because these new decumbent populations are not assignable to either *F. decumbens* or *F. californicum* based on leaf length, trichome ray length and flower color, their taxonomic status remains undetermined.

A fundamental question concerning the conservation management of *F. decumbens* is whether it represents a distinct taxon or is merely an ecotype of *F. californicum*, as implied by the transfer of *F. decumbens* to *F. californicum* by Munz (1968). Likewise, the management of the newly identified decumbent populations depends on whether they are assigned to *F. californicum*, *F. decumbens*, or a new taxon. This problem was emphasized in the recovery plan for Gabbro Soil Plants of the Central Sierra Nevada Foothills (USFWS 2002), which recommended that "the decumbent *Fremontodendron* within Nevada and Yuba Counties should be secured and protected unless they are determined not to be the listed [*F. decumbens*]." Since morphological evidence has not provided a clear answer to the species placement of the decumbent plants in Yuba County, molecular markers were used to clarify the genetic relationships among *F. decumbens*, *F.*

californicum, and the decumbent population near Brownsville, Yuba County, and so provide a stronger basis for conservation decisions affecting these taxa.

METHODS

Leaf Sample Collection

The localities of the five populations are shown in Fig. 1. *Fremontodendron decumbens* leaf samples were collected from eight shrubs in the Pine Hill (PH) area of El Dorado County, all of which were within 150 m of each other. A further nine plants were sampled from a nearby population approximately 0.8 km north of Pine Hill (BLM), all within 20 m of each other. *Fremontodendron californicum* leaves were collected from eight plants on Tollhouse Road, Fresno County (THRD) and seven plants on Highway 168, Fresno County (HWY168); the sites are approximately 2.5 km apart. The "Yuba County" leaves were sampled from nine shrubs from the population near Brownsville, Yuba County (Yuba) mentioned in the introduction. Four additional samples were obtained from shrubs that were grown in Canberra, Australia, from seed of the same Yuba County population, accessed as Commonwealth Plant Introductions (CPI 140372, CPI 140375, CPI 140376, and CPI 140378). Leaves were preserved using silica gel or lyophilized prior to DNA extraction.

DNA Extraction and AFLP Procedures

Total genomic DNA was extracted using 10 mg of dried leaf tissue ground to a fine powder using 3 mm tungsten carbide beads in a Retsch MM300 mixer mill and extracted using the Qiagen 96-well DNEasy Extraction Kit (Qiagen, Melbourne). Amplified marker length polymorphism (AFLPTM) templates were prepared and selectively amplified using three AFLP primer combinations (*E*-AGC:*M*-CAT, *E*-AGG:*M*-CTC, *E*-AGG:*M*-CTG) following Brubaker and Brown (2003). The *Eco*I-ANN primer was end-labeled with [³²P]-dATP and the AFLPs

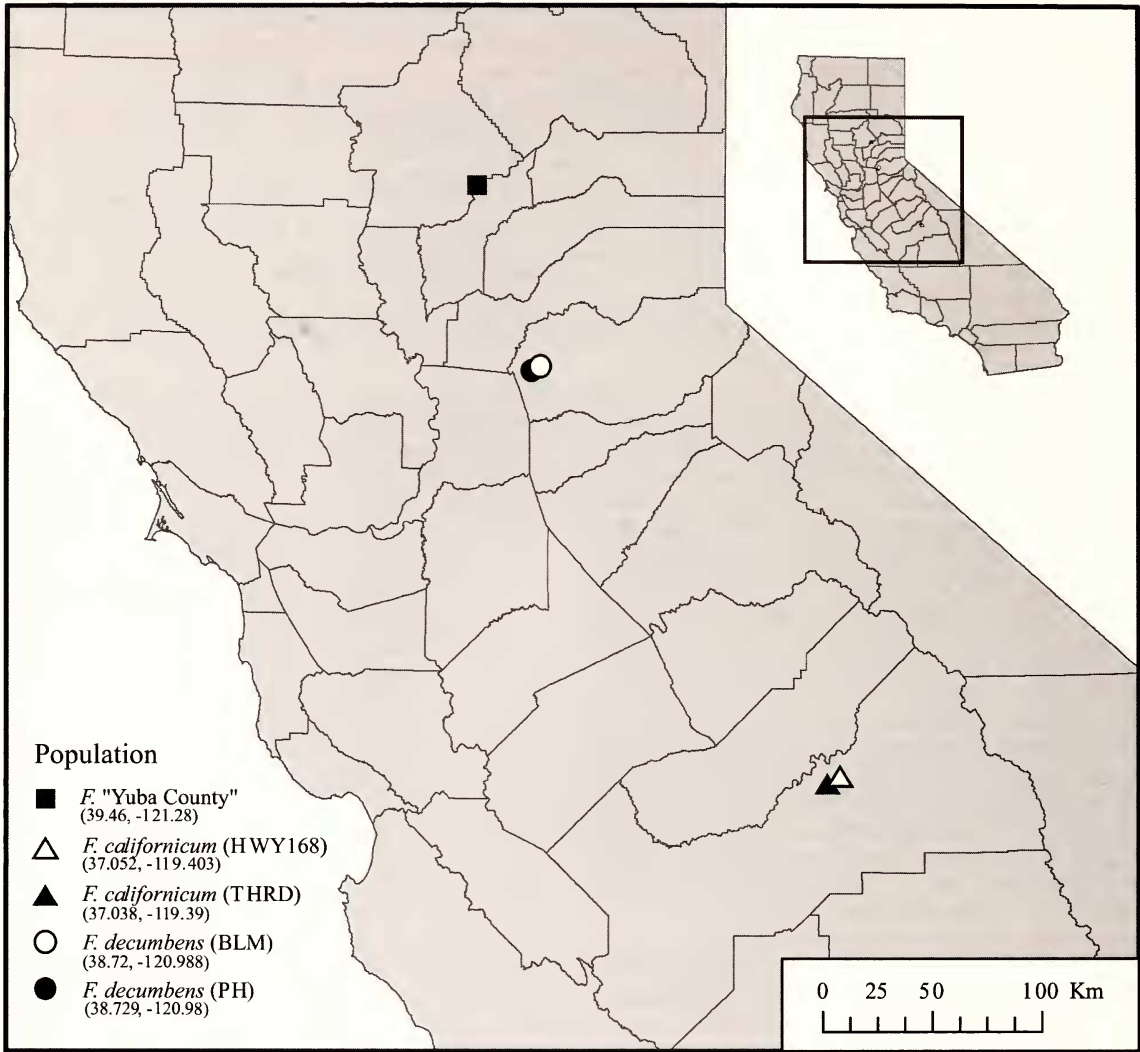


FIG. 1. Location of *Fremontodendron californicum*, *F. decumbens*, and *F.* "Yuba County" populations sampled.

were resolved on 6% denaturing polyacrylamide gels run at 50 watts using 1X Tris-Taurine-EDTA (TTE) buffer (following Brubaker and Brown 2003). The polyacrylamide gels were dried to the short plate and exposed to BiomaxMR (Kodak) film for one to four days. Autoradiograms were scored for the presence/absence of a total of 66 polymorphic AFLP loci that were numbered sequentially from largest to smallest.

Data Analyses

Allele frequencies were calculated with AFLP-SURV Version 1.0 (Vekemans 2002) using a Bayesian approach that assumed Hardy-Weinberg (H-W) genotypic proportions with non-uniform prior distribution of allele frequencies (Zhivotovskiy 1999) and used to estimate average heterozygosity (H_j) which is analogous to gene

diversity (H_e), the proportion of polymorphic loci (P), expressed as a percentage at the 5% level as well as total gene diversity (H_t) and genetic diversity within populations (sites) (H_s). Genetic differentiation among populations was assessed using Wright's F_{ST} following Lynch and Milligan (1994) with the confidence intervals (CI) estimated following 1000 permutations. Estimates of the pairwise relatedness coefficients between individuals (r) were calculated using AFLP-SURV following Lynch & Milligan's Taylor expansion (Lynch and Milligan 1994) and the relatedness matrix produced was used to extract and plot the first two principal coordinates (PCO). Nei's (1978) genetic distance (D) was calculated between population pairs using POPGENE (Yeh and Boyle 1997) and a UPGMA dendrogram was constructed using NTSYS 2.11X (Rohlf 2005).

TABLE 2. GENETIC DIVERSITY IN *FREMONTODENDRON* POPULATIONS ASSESSED USING AFLPs. (n = number of individuals genotyped; P = percentage of polymorphic loci; H_c = Gene Diversity)

Taxon	Population	n	P	H_c	Private Alleles
<i>F. decumbens</i>	BLM	9	22.2	0.120	1
	PH	8	75.9	0.274	
<i>F. californicum</i>	THRD	8	74.1	0.228	2
	HWY	7	63.0	0.222	
<i>F.</i> "Yuba County"	Yuba	13	72.2	0.284	5

Differentiation among populations and species was also assessed using STRUCTURE Version 2.1, which is a model-based clustering method for inferring population structure (Pritchard et al. 2000). In the first stage of the analyses, the two *F. californicum* and the two *F. decumbens* populations were analyzed without using any prior information to determine the most likely number of sub-populations (K). Following this, individuals within each species were reassigned to their most likely sub-population and the origin of the "Yuba County" individuals was inferred relative to this genetic background. All analyses were conducted using a 30,000 burn-in and 300,000 runs and were replicated five times.

RESULTS

Levels of genetic diversity varied among the populations (Table 2). The *F. decumbens* BLM population had the fewest polymorphic loci ($P = 22.2\%$) and lowest gene diversity (H_c , 0.120) of all the populations sampled. Levels among the other populations were similar although the Yuba County population had the highest gene diversity (0.284), possibly reflecting the larger sample size of this population. Unique alleles were present in PH (1), THRD (2) and Yuba (5). The low level of genetic diversity in BLM arose from the fact that there were only three multilocus genotypes in the population. In contrast, all individuals in the other three populations were genetically dissimilar. Population differentiation indicated that most of the total genetic diversity ($H_t = 0.307$) was apportioned within sites ($H_s = 0.226$), with significant genetic differentiation among populations ($F_{ST} = 0.263$, $p < 0.001$).

The first principal co-ordinate placed the two *F. decumbens* populations in negative space while the *F. californicum* populations were in positive space (Fig. 2A). Almost all the Yuba County individuals ordinated in positive space with the *F. californicum* individuals along the first principal coordinate (Fig. 2A). As predicted by the F_{ST} value, genetic distances among the five populations were also high and long branch lengths separated *F. californicum* and *F. decumbens* in the UPGMA topology (Fig. 2B). The Yuba County individuals clustered with the *F. californicum* populations (Fig. 2B).

The genetic differentiation between the *F. californicum* and *F. decumbens* populations evident in the principal coordinates analysis was also apparent in the initial STRUCTURE analysis. The most likely number of inferred gene pools was four rather than two [mean $\ln P(D)$ of -473 versus -625]. Both taxa were clearly divided into two gene pools that were largely congruent with their population of origin (Fig. 3A). When the origins of the Yuba County individuals were inferred relative to this genetic background, it emerged as a genetically distinct element, a result that correlated with the high number of unique alleles present in this population (Table 2). Nonetheless, there were obvious genetic inputs to the Yuba County population from both *F. californicum* and *F. decumbens* (Fig. 3B), although the level of this influence varied substantially among individuals. The influence of *F. californicum* appeared to be greater than that of *F. decumbens*, a result consistent with the principal coordinates analysis, where the Yuba County individuals ordinated with *F. californicum* along the first axis (Fig. 2A). A structure analysis, in which no population information was used, produced a congruent result (i.e., there was unambiguous support for five distinct populations).

DISCUSSION

This study was prompted by uncertainty regarding the taxonomic rank of decumbent *Fremontodendron* populations and the consequences of this for their conservation management. As evident in Table 1, a number of diagnostic morphological features (in addition to habit) differentiate *F. decumbens* from *F. californicum*. The genetic evidence presented here using measures of genetic distance (Fig. 2B) and population structure (Fig. 3A) also clearly separates the two species. A taxonomic treatment that merges these populations in a single species belies the genetic, morphological, and ecological discrimination between the two taxa and weakens the case for the conservation of the *F. decumbens* populations of El Dorado County.

Species with restricted distributions, such as *F. decumbens*, generally exhibit lower levels of genetic diversity than their more widespread

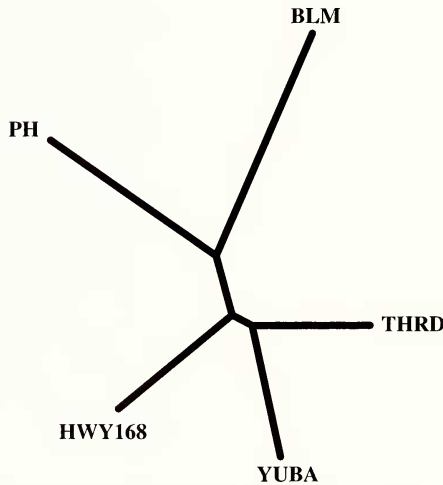
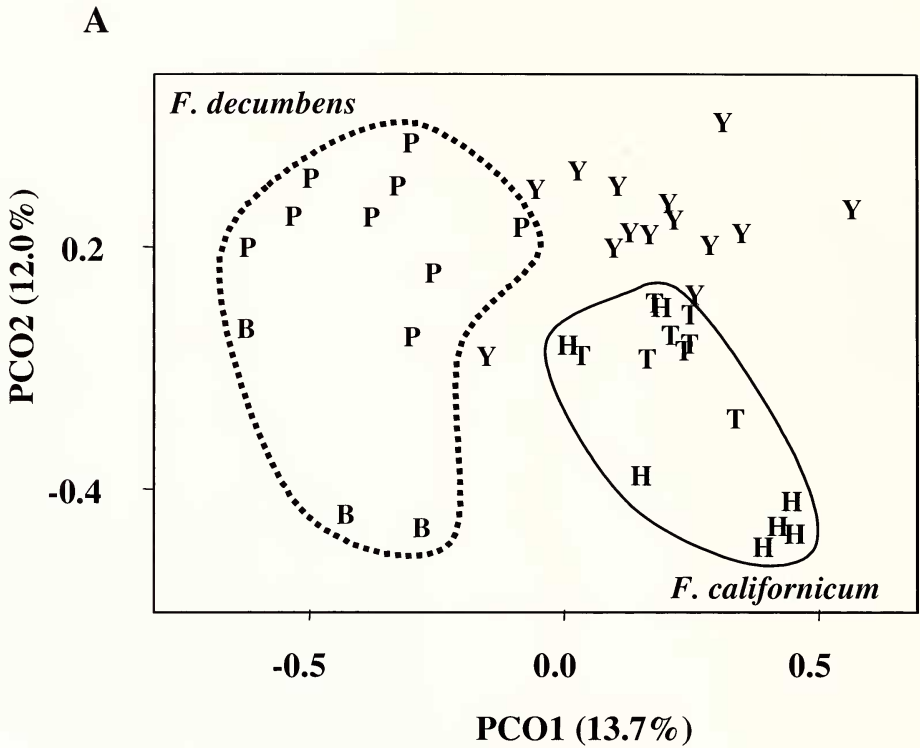


FIG. 2. A. Principal coordinates analysis of *F. decumbens* (P = PH, B = BLM), *F. californicum* (H = HWY168, T = THRD) and Yuba County (Y) individuals based on Lynch and Milligan's (1994) relatedness matrix. B. UPGMA topology based on Nei's (1978) genetic distance (*D*).

counterparts (Hamrick and Godt 1989; Gitzen-danner and Soltis 2000), but this is not always the case and probably reflects the myriad of factors that can contribute to rarity (Karron 1987; Fiedler and Ahouse 1992). The *F. decumbens* PH population had the highest levels of genetic diversity of the two species indicating it is not

genetically depauperate with respect to its wide-spread congener. However, further sampling of *F. californicum* is necessary to accurately quantify the differences in genetic diversity between the two species. In contrast, plants of the other *F. decumbens* population (BLM), which were within 20 m of one another, had substantially lower

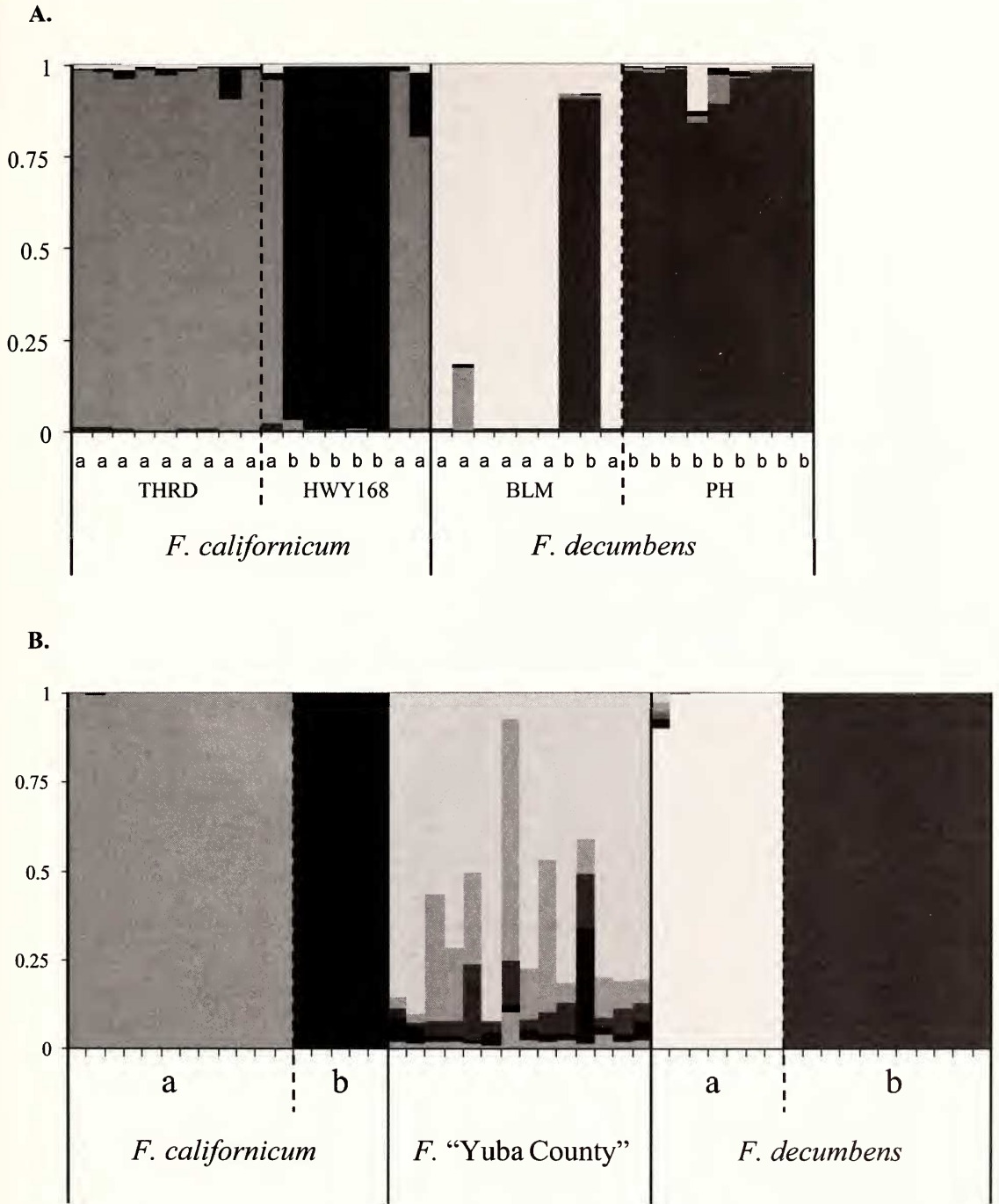


FIG. 3. Pictorial representation of the genetic structure among the five *Fremontodendron* populations generated using STRUCTURE (Pritchard et al. 2000). The different shades represent genetic subsets of the sampled populations. A. An analysis without the Yuba County individuals suggests that there are two distinct gene pools within *F. decumbens* and within *F. californicum*, designated "a" and "b", respectively. B. Based on the first analysis, individuals were re-designated by their respective gene pools and the origins of the Yuba County individuals were assigned relative to *F. decumbens* and *F. californicum*.

levels of genetic diversity and a high level of relatedness among individuals. This may indicate a recent founder event, vegetative reproduction, or that genetic erosion and high levels of

inbreeding have occurred. Although reduced heterozygosity can be deleterious to population fitness (Reed and Frankham 2003) and compromise the long term persistence of populations

through poor seed set, smaller seed, slower germination response, poor seedling survival, and reduced reproductive capacity (Buza et al. 2000; Oostermeijer 2000; Young et al. 2000; Tomimatsu and Ohara 2003), this is not always the case, particularly for self-fertile species. While further genetic and demographic investigation of the BLM population is warranted, habitat loss is probably the most critical short-term process to manage.

The taxonomic status of the more recently discovered decumbent populations found in Yuba and Nevada Counties is more difficult to resolve. They do not have the distinctive correlated characters of *F. decumbens* and on other morphological grounds would fall within the range of variation of *F. californicum*, and if it were not for their decumbent habit they would have been so treated. The genetic data for the Yuba County individuals is similarly ambiguous. In the ordination analysis, the Yuba County individuals grouped within a larger cluster of *F. californicum* individuals along the first axis, and there were genetic inputs to the Yuba population from *F. californicum* and *F. decumbens*. While a number of hypotheses could be formulated to explain these relationships, the most likely scenario is that the Yuba County population (and by inference the Nevada County population) is derived from an historical hybridization between *F. californicum* and *F. decumbens*. This could explain, in part, the higher levels of genetic diversity observed in the Yuba county population relative to *F. californicum* and *F. decumbens*. Hybridization is a common event in angiosperms and estimates of natural hybridization among plant taxa range from 6–22% worldwide (Ellstrand et al. 1996), primarily due to the semi-permeable nature of plant reproductive barriers (Harrison 1993). Hybridization can lead to increased intra-specific genetic diversity, the origin and transfer of genetic adaptations, the origin of new ecotypes or species, and the reinforcement or breakdown of reproductive barriers (see Rieseberg 1997). The unequal contributions of *F. californicum* and *F. decumbens* to the Yuba County population could be explained by subsequent backcrossing to the more widespread *F. californicum*. That the genetic makeup of the Yuba County individuals can not be ascribed simply to *F. californicum* and *F. decumbens* suggests that the actual parental populations may not have been sampled (particularly for *F. californicum*), or that the hybrid populations have existed long enough that backcrossing and directional selection has substantially shifted gene frequencies.

Our results do not support definitive conservation recommendations regarding the Yuba and Nevada County populations, but the presence of unique alleles in the Yuba County gene pool

indicates that this population should be conserved until further data are available. The morphological similarity and spatial proximity between the Yuba and Nevada County decumbent populations predict they will be genetically similar. When the single Nevada County individual that we had access to was included in a separate principal coordinates analysis (not presented), it fell within the Yuba County cluster, but this population needs to be sampled more thoroughly before any robust conclusions can be made. As noted above, there is a strong possibility that the Yuba and Nevada County populations originated from hybridization. The conservation of hybrid populations is controversial since introgression can lead to species extinction (Allendorf et al. 2001). With increased fragmentation of natural habitats around the world, however, once rare hybrid events may become more common place and could threaten species integrity. The challenge for conservation managers is to determine whether hybridization events are natural or anthropogenic (Allendorf et al. 2001), and whether they pose a real threat. Like their congener, *F. decumbens*, the distribution of the other northern decumbent populations appears to be associated with gabbro or ultramafic substrates. It is hard to envision how one or more anthropogenic hybridization events would result in the apparent substrate-specific distribution observed. If the decumbent populations share adaptation to these unusual substrates, this would be another indicator of a shared genetic heritage. Any proposed reintroduction of *F. decumbens* should not use the Yuba and Nevada County populations as sources of seed. Resolving their taxonomic status will require a more extensive sampling of *F. californicum* populations.

While an understanding of the levels and patterns of genetic diversity is an integral component of species management, ecological and evolutionary interactions should also be considered (Holsinger and Gottlieb 1991). *Fremontodendron decumbens* is pollinated by native bees (Boyd 1994), has seeds which are dispersed by ants (Boyd 1996) and suffers high reproductive attrition from insects and rodents (Boyd 2003). Furthermore, *F. decumbens* and the other decumbent populations are components of plant communities that have varying reestablishment strategies following fire (Marsh and Ayres 2002). These mutualistic and community relationships highlight the importance of conserving these populations as integral elements of the Sierra Nevada gabbro flora (USFWS 2002).

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